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ROLE OF METAGENOMICS FROM TRADITIONAL MICROBIOLOGY TO GENOMIC WORLD

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ABSTRACT:

The study of microbial genomes through direct extraction, DNA cloning, and a panel of microorganisms is known as metagenomics.We all know that microbes are omni present in the world. Using microbial techniques for organism identification will be more helpful in understanding all the microbes that make up diversity. Microbiologists usually do all the laboratory works for the identification and characterization of the particular organisms based on their morphology. But this method of identification is not sufficient for the complete identification of microbes in the particular diversity. To fulfil this issues, we microbiologists need a better method to identify all the microbes present in the entire environment. Metagenomic methods play a major role to identify all the genes present in the particular community by means of both functional based and sequence-based screening. This method also plays a way to know the taxonomic understanding with gene profiling.Shotgun sequencing was supplanted in the area of sequencing by high-throughput third-generation sequencing (TGS) and next-generation sequencing (NGS) technologies. The rapid identification of pathogenic microorganisms has been shown to be an advantage by NGS and TGS. The effectiveness of petrographic profiling and genetic prediction of microbiological species will be improved by the application of new algorithms. New bioactive compounds, functional microbial genes, and microbial metabolites were studied using functional metagenomics. In this review, the main applications of metagenomics in microbiology can be elucidated.

KEY WORDS: Metagenomics, NGS, TGS, Functional Screening, Sequence Based Screening, Microbiology, Enzymes

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INTRODUCTION:

Researchers used traditional methods for the identification of cultured organisms but it has several disadvantages (Kellenberger, 2011) in isolating the particular gene.The metagenome, also known as the environmental genome of microorganisms, is the determination of the total genome found in nature, according to Handelsman et al. (1998). Metagenomics is a powerful approach to studying microbes in environments as diverse as soil, water, and the human gut. By directly analyzing the genetic material from samples, this technique enables scientists to comprehend the variety, functionality, and interactions of microorganisms without having to grow them in a lab.This can be done through both functional and sequence-based analyses, providing valuable insights into the microbial communities present in these environments. Research in metagenomics produced more novel genes with unique applications and all-important fields of microbiological sciences. Metagenomics has, had a significant impact across various life sciences domains. Its applications span fields like ecology, microbiology, biotechnology, and medicine. The shift from traditional microbiology to metagenomics required the development of new academic disciplines and specialized expertise due to its unique methodologies and data analysis techniques. This change has allowed researchers to better understand microbial communities and their functions in different environments.Here, we'll talk about the impact high-throughput sequencing technologies have had on microbiology and bioinformatics as well as the computational challenges the DNA sequencing revolution has brought about.Metagenomics involves the integration of different computational methods as it involves the collection, processing and extraction of valuable biological information from many samples and complex datasets.This interdisciplinary approach is crucial for effectively analyzing the substantial amount of genetic data present in metagenomic samples and for improving our comprehension of the make-up and purpose of microbial communities. Although bioinformatics requires a high level of expertise, life scientists need to understand it (Escobar-Zepeda et al., 2015). The traditional approach to studying bacteria in human health has largely revolved around identifying and treating pathogens with antibiotics. However, the emergence of antibiotic resistance and the movement of people around the world have required a paradigm shift. The advent of next-generation sequencing (NGS), sometimes referred to as high-throughput sequencing, has made it possible to control microbes more thoroughly for the benefit of human health.This technology allows researchers to analyses microbial communities comprehensively, providing information that goes beyond simple pathogen identification and extends to understanding the overall microbial ecosystem and its impact on health.The composition of the microbiome varies between different populations due to factors like genetics, mode of birth, diet, location, age, and exercise. individual. This intricate microbiome is crucial for the immune system, diabetes, atherosclerosis, and interactions with foreign substances, among other aspects of human health.

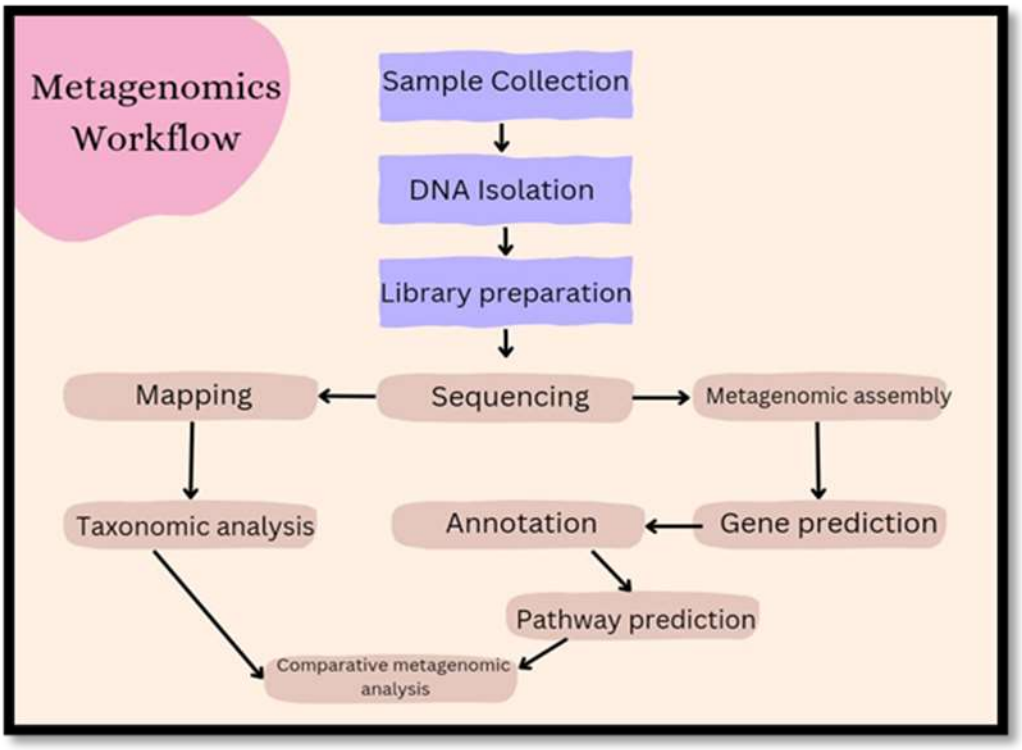
HISTORY OF METAGENOMICS:

The first report on microorganisms provided by changed the way microbial communities are studied today. The characterization of Leeuwenhoek and the organisms in his mouth in 1676 (Schaerbeek, 1959) used available molecular methods.At first, scientists tried to distinguish between these "invisible" species. As a result, they began by cultivating and isolating bacteria,

much like Robert Koch, in order to count and visualize them using solid food sources like potato slices or gelatin.Ultimately, these scientists were able to understand the physiology of microorganisms using isolation procedures (Blevins and Bronze, 2010).

The main tool for researching microorganisms and their interactions was quickly the microscope. The resolution of microscopic techniques has significantly improved thanks to the development of useful staining techniques like Gramme, Ziehl-Neelsen, and Schaeffer and Fulton .In 1977, Carl Woese pushed for the use of ribosomal RNA genes as molecular indicators to classify life (Woese and Fox). The way microorganisms are studied and classified has changed as a result of the combination of this concept and Sanger's automated sequencing technique . A few decades later, improvements in molecular techniques made it possible to characterize microbial diversity and make the "new wild world" of bacteria available for study. rRNA gene cloning and sequencing, length polymorphism, restriction fragment analysis (RFA), denaturing gradient gel electrophoresis (DGGE and TGGE).

METAGENOMIC WORKFLOW:

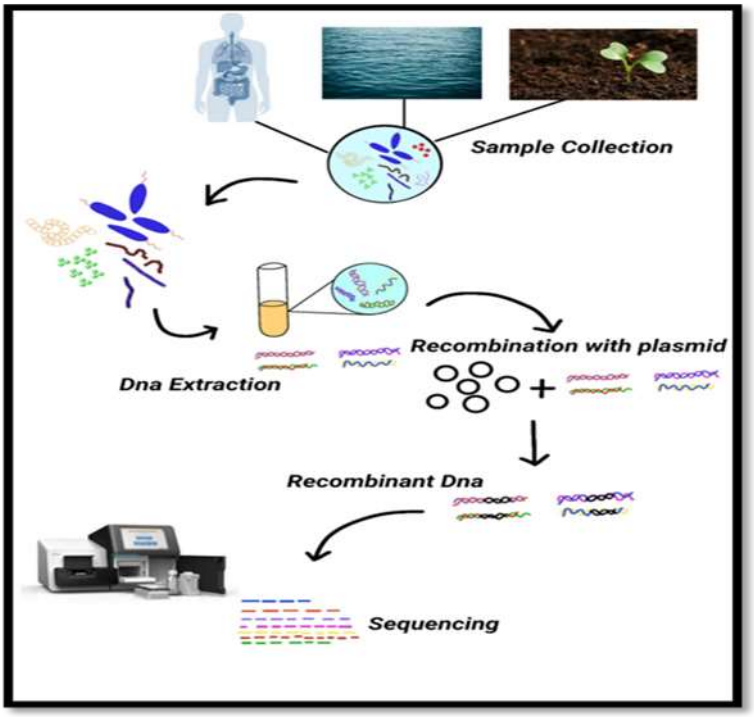


METHOD OF METAGENOMICS:

We specifically focus on sample single reconstruction workflow, DNA sequencing, assembly, annotation. There are several steps involved in the actual DNA sequencing process. These steps include sampling, DNA extraction, and purification. Although these steps are beyond the scope of this article, they are important to highlight (Pollock et al., 2018). Avoiding contamination and bias caused by DNA extraction and degradation during processing and

storage is particularly important. Metagenomics is built on gene cloning.The steps below constitute the basic metagenomics workflow. The first step consists of extracting and enriching all genes from the surrounding microbial samples. To create a metagenomic library, the vector is transformed into host bacteria after the genes are cloned. Then, the metagenomic library was examined and screened.Metagenomic library generation and screening as well as metagenomic DNA extraction are important.Since 99% of environmental microorganisms are not culturable, a typical approach is to first culture the bacteria before isolating the DNA. Collecting a sizable portion of the total DNA of the nearby microorganisms is the first and most important step in the creation of a metagenomic library. The process of obtaining metagenomic DNA involves two important steps. To begin with, all of the sample's microbes' genes must be extracted.Secondly, to preserve the fragment's integrity and purity. Sample collection must adhere to precise protocols in order to recover as much DNA as feasible from environmental bacteria while preserving big DNA fragments. There are two types of extraction: direct extraction and indirect extraction.

STEPS IN METAGENOMICS:



EXTRACTION OF METAGENOMIC DNA:

To construct superior metagenomic libraries, extricating add up to DNA of natural microorganisms in tall concentrations and huge parts is to begin with and first. There are two fundamental focuses within the metagenomic DNA extraction prepare. To begin with, separate qualities from all microorganisms show within the test. Moment, keep up the cleanliness of the fix. In order to protect large DNA parts and extract as much DNA as is possible from natural microorganisms, strict inspection techniques must be followed. There are two types of DNA extraction strategies: coordinate extraction and roundabout extraction. In order to specifically disrupt microorganisms and release microbial DNA from the test, coordinate extraction employs

physical (such as freeze-thaw) and chemical (such as expansion of protease or SDS) strategies .1 to 50 kb of DNA are extracted using the coordinate extraction method.The coordinate extraction strategy is straightforward and compelling but has moo virtue. The backhanded extraction method requires to begin with confining the microbial cells and after that extricating the DNA employing a tender strategy. DNA gotten by the backhanded extraction strategy is exceptionally immaculate and appropriate for the extraction of DNA with sizes from 20 to 500 kb. Backhanded extraction strategies are awkward and wasteful, so a few microbial DNA may be misplaced. Test measure depends on microbial concentration. In cases of tall microbial thickness, such as stool tests, as it where a rectal light is required. A huge number of water tests were collected and concentrated through channels to consider marine microbial communities, which speak to moo microbial densities. Tests must be cleaned. For illustration, humic acids found in soil are frequently firmly bound to DNA; subsequently, it must be evacuated amid test planning (Daniel, 2005). When considering microorganisms in people or creatures, DNA defilement must be expelled from the test and have. For frail DNA tests, enhancement at the cellular and hereditary level can be performed after DNA part segregation, but may present a few predisposition (Probst et al., 2015). Common hereditary improvement strategies incorporate steady isotope examining (Taste), deletion-subtraction hybridization (SSH), DNA tests, etc.Building distant better;a much better;a higher;a stronger;an improved"a Much better Metagenomic Library Segregation of tall concentrations, profoundly divided microbial DNA from the whole medium is the primary, first, and most critical step. There are two primary focuses within the metagenomic DNA extraction handle. To begin with, confine qualities from all microorganisms show within the test. Moment, keep up the cleanliness of the fix.

METAGENOMIC DNA PURIFICATION AFTER ISOLATION:

Humic corrosive is the most prevalent DNA contaminant that is not connected to the soil in the case of soil living spaces. Their removal enables the use of PCR, invert translation, absorption, or ligation. The brown color of the extricate serves as an example of the co-purification ability of humic acids, which show in soil to have charge properties comparable to DNA (Sharma et al., 2007).Because they exhibit absorption at wavelengths of 260 nm and 230 nm (the latter used to evaluate DNA), corrosive humic substances also damage DNA analysis (Sharma et al., 2007). Depending on the soil's structure, the DNA filtration processes can be quite complex or very simple. Natural matter as a whole, other potential chemical inhibitors (such as metal), and substances like clay.

SAMPLING & METADATA:

Examining can be of specific significance for the quality of the information gotten as well as for the elucidation of the comes about. This is often considered a critical step when employing a metagenomic approach since the test had may not be agent in measure (Thomas et al., 2012). A point by point depiction of the natural setting and the strategies utilized is vital to compare thinks about and comes about. Organizing assorted and complex information that clients can openly find, effortlessly get it, and analyze agreeing to their inclinations is getting to be progressively vital (Barret et al., 2012).Handelsman and colleagues (Metagenomics Committee: Functional Challenges and Applications, National Research Council, 2007) recommends careful reflection

on sampling strategies and variability of testing methods. The metadata collected gives data almost the source of the test as well as when and beneath what conditions the test was collected.

SEQUENCING:

It is now possible to take into account the unique genetic characteristics of the uncultured portion of the host-associated microbial community thanks to advances in DNA sequencing and bioinformatics. For example, amplicon sequencing is the most widely used method to characterize variations in the microbiome. In this case, DNA is extracted from all cells tested and the community is examined (e.g. using water, soil or tissue biopsies). The overall sequence of clones with phylogenetic relicts shows that the sorted group is most likely to be the origin of the DNA element that could be included in the sequence-based analysis. On the other hand, Roumpeka DD et al., random sequencing can be performed and once the level of interest is determined, phylogenetic struggles can be examined in neighboring DNA to establish relationships developmental network between genes and useful qualities.

FUNCTIONAL METAGENOMICS:

A capable however challenging approach to metagenomic investigation is the useful assurance of clonal expression. Victory requires steadfast translation and interpretation of the gene(s) of intrigued and discharge of the quality item on the off chance that testing or investigation requires it to be extracellular. Useful examination has recognized modern anti-microbials (Courtois, Gillespie, MacNeil et al.), anti-microbial resistance qualities the Na (Li)/H transporter and corrupting chemicals. The control of this strategy lies within the reality that it does not require distinguishing proof of qualities of intrigued by grouping examination, making it the as it were metagenomics approach that can distinguish totally unused classes of qualities for capacities. modern or particularly known.

RESOLUTION AND METAGENOMIC ANALYSIS TECHNIQUES:

Short-term sequencing is rapidly being replaced by third-generation sequencing (TGS) and efficient next-generation sequencing (NGS).

METAGENOMIC DETERMINATION TEST PLANNING:

DNA libraries can be used to sequence metagenomic DNA gotten by cloning the target quality or specifically from tests. The immaculateness and substance of the DNA must be guaranteed some time recently sequencing.

APPLICATIONS OF METAGENOMICS IN MICROBIOLOGY:

Application	Function	Reference
Environmental pollution reduction and control	1.Metagenomics plays an imperative part in bioremediation. 2. Through the development and choice of modern microbial strains with tall	(Lammle et al., 2007). (Krüger et al., 2003)

	corrupting productivity, wide appropriateness, and steady expression, metagenomics can help within the investigation of novel useful microorganisms and qualities.	
Dischargeremediation	1. Water microorganisms are subjected to metagenomic investigate, which bolster organic nitrogen expulsion forms and move forward natural phosphorus evacuation. 2.Microbial electrochemical frameworks (MES) utilize anode-enriched electroactive microbes to get power straightforwardly from natural squander.	(Lammle et al., 2007). (Krüger et al., 2003)
Metagenomics Are Utilized within the Therapeutic Conclusion of Pathogenic Microorganisms	1.Metagenomic discoveries recommend that the intestine microbiome is included in a few illnesses, counting non-alcoholic greasy liver illness, immune system infections, and tumors. 2.Metagenomics can be utilized to distinguish medicate resistance qualities in pathogens and, to screen episodes of irresistible illnesses in clinics and communities.	(Aron-Wisnewsky et al., 2020) (Svoboda, 2021) (Andrews et al., 2021)
Escherichia coli have designing for proficient metagenomic chemical revelation	1.The developing request to move the center from petrochemical to biotechnology-based businesses has extended the utilize of chemicals. 2.Until presently, most	Reia Hosokawa-Okamoto and Kentaro Miyazaki

	mechanically critical chemicals are of microbial root. 3.In any case, right presently less than 1% of common organisms can be created inside the investigate office.	
Quorum-sensing and plant-pathogen interactions	1.In spite of the fact that metagenomics may be a modern instrument within the field of plant-microbe intelligent, the strategy has as of now driven to noteworthy progresses. 2.Distinguishing plant pathogens that have not yet evolved or represent the microbiota of plants and rhizomes are two outstanding facts that can lead to something much, much better; superiority; a stronger one; improved representation of agricultural soil quality, for example in the case of prediction of soil diseases.	Denis Faure, Mélanie Tannières, Samuel Mondy and Yves Dessaux
Finding novel qualities and microbial pathways	1. Utilizing practical metagenomics of the microbial characteristics of the human digestive system, Tasse et al discovered novel CAZymes. 2. Intestinal microbes made notable advances in these abilities.	Tasse L, Bercovici J et al (2005)
Investigating antibiotic-resistance genes	1. The development of the human gut-associated resistome has been prompted by the discovery that several human commensal microbiotas possess different antibiotic-resistance traits (ARGs). Subsequent analyses have nearly entirely	Salyers AA

	supported this finding.	
Synergism	1. Many of them live in extraordinary structures in have tissues, frequently in unadulterated or exceedingly improved culture, making them perfect candidates for metagenomic investigation since microbes can be effectively recognized from have tissues and other microorganisms.	Preston et al (2015)
Small Molecules	1.Conventional anti-microbial screening for bacterial development inhibitory particles driven to the revelation of anti-microbials in metagenomic libraries	Brady et al (2001)
Biogeochemical Cycles	1. Mine spillage of acid. Metagenomics' exciting promise is to provide a communal evaluation of metabolic and biogeochemical activity. 2. A community's food and imperativeness budget can be protected by creating models of how life forms share the workload based on an analysis of each individual's distinct capacities.	Baker et al (2003)

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